What you see is not what you get! DNA barcoding is helping scientists unveil nature's most hidden diversity

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Any chef in town knows what *Lutjanus campechanus*, also known as the red snapper, looks like. It is a pricey, medium-sized, light-red fish that weighs about 10 pounds. You may enjoy it fried, baked, broiled, poached, or sautéed. But what the chefs don't know is that about 77% of red snappers sold in the United States are actually a mix of different species that includes some not-so-appreciated and rare reef fish (1). That's because some snappers look very much alike, making it difficult to sort them based on appearance. Besides being a possible theft against consumers who pay too much for ordinary reef fish, the mislabeling results in deceptive stock sizes of *L. campechanus,* with several implications for resource management.

Over two centuries ago, the great taxonomist Carl Linnaeus introduced the binomial species nomenclature that is still in use today. Focused mainly on morphology, Linnaeus' pioneer work was a milestone toward a classification system of the species. In 1942, the ornithologist Ernst Mayr proposed the biological species concept (BSC) (2) by which a species is defined as a group of organisms reproductively isolated. The BSC considers the organism's behavior, geographical location, reproductive attributes, genetic data, ecology, and physical appearance. Now scientists want to add a new wrinkle to the species definition: a DNA bar code.

According to Paul Hebert, one of the fathers of DNA barcoding, both phenotypic plasticity and genetic variability in a given key character can lead to a faulty classification. Additionally, most morphological keys are particular to a life stage or gender, preventing many organisms from being identified this way. A barcode, in contrast, can be applied to all stages of life (including eggs) and used for routine identification as well as detection of hidden species, with no expertise required.

Scientists believe that mitochondrial DNA is the best choice for DNA barcoding in animals, for many reasons. First, mitochondrial DNA is maternally inherited, which avoids recombination among individuals of the same species. Second, the low frequency of DNA deletions and insertions makes sequence alignments of different species easier because abrupt gaps are rare. Third, mitochondrial DNA is present in many copies in the cell and therefore easier to detect.

Recent studies (3) have shown that a 650-bp stretch of the mitochondrial cytochrome c oxidase I gene (*COI*) is very powerful in discriminating species and phylogeographic groups within species. Scientists attribute that to *COI* fast evolutionary rate and high incidence of base substitutions in third-position nucleotides. The existence of robust primers that enables routine PCR of the *COI* locus in most species has also contributed to make *COI* the number one choice for DNA barcoding.

In reality, any locus that has evolved fast and is abundant in the cell could be used for this purpose. In the past, scientists have inferred phylogenetic relationships using the 12S rRNA and 16S rRNA mitochondrial genes (4-6). Other possible choices include genomic rRNAs such as 18S and 28S, although their slow rates of divergence might represent a problem

for species delineation. The real power of DNA barcoding lays on the ability of using the same locus to classify all species.

Hebert and his team (7) have used the *COI* system to identify 200 species of lepidopterans as well as several new specimens. More recently, the same group was able to identify 1,500 species by using this system (8). In another study (9), the application of DNA barcoding to a museum collection revealed that what has been known as the *Astraptes fulgerator* is actually composed of 10 previously unknown species. More recently, scientists have tested 260 species of North America birds and found that each species has a unique *COI* DNA barcode (10). Several other projects are underway, including a project to barcode Costa Rica's plants and Fish-BOL, an initiative that will collect 15,000 marine and 8,000 freshwater species, with the hope of assembling DNA barcodes for all fish.

The *COI*-based identification system does not work with all species, though. In some groups that have evolved apart recently, such as the stony corals and the cichlid fish, the 650-bp stretch has not accumulated enough variation. In other groups such as amphibians, *COI* primers have shown lower success rates because of highly variable *COI* priming sites present among groups and closely related species (11).

In the next 20 years, The Consortium for the Barcode of Life (CBOL), an international initiative that aims to accelerate compiling of DNA barcodes of known and newly discovered species, expects to have a *COI* profile for most of the estimated 5–10 million animal species on the planet. Using DNA barcoding, it will be possible to identify a species using as little as a single cell, allowing the detection of minute amounts of undesirable or regulated species in processed foods. Other applications include the identification of bird species that strike aircraft and of mosquitoes' eggs and larvae, which represent health threats to people around the world. DNA barcoding might also help protect endangered and threatened populations and prevent the mislabeling of commercial species.

Scientists hope that DNA barcoding will be a master key in precisely identifying every single species on the planet, and that includes the pricey and so-called red snapper sitting on your dinner plate

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Box: DNA Barcoding

Using NCBI resources to find *COI* DNA sequences

For this tutorial, you will need the latest version of Flash player [http://www.macromedia.com/go/getflashplayer] installed on your computer.

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